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Review

The evolution of developmental mechanisms

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Abstract

Over the past two to three decades, developmental biology has demonstrated that all multicellular organisms in the animal kingdom share many of the same molecular building blocks and many of the same regulatory genetic pathways. Yet we still do not understand how the various organisms use these molecules and pathways to assume all the forms we know today. Evolutionary developmental biology tackles this problem by comparing the development of one organism to another and comparing the genes involved and gene functions to understand what makes one organism different from another. In this review, we revisit a set of seven concepts defined by Lewis Wolpert (fate maps, asymmetric division, induction, competence, positional information, determination, and lateral inhibition) that describe the characters of many developmental systems and supplement them with three additional concepts (developmental genomics, genetic redundancy, and genetic networks). We will discuss examples of comparative developmental studies where these concepts have guided observations on the advent of a developmental novelty. Finally, we identify a set of evolutionary frameworks, such as developmental constraints, cooption, duplication, parallel and convergent evolution, and homoplasy, to adequately describe the evolutionary properties of developmental systems.

Keywords: Evolution and development; Fate maps; Asymmetric cell division; Induction; Developmental genomics; Competence; Redundancy; Positional information; Determination; Lateral inhibition; Gene networks; *C. elegans*; *Volvox*; Cavefish; Threespine stickleback fish; *Drosophila*; *Tribolium*; Mouse; Phylogeny; Satellite systems; Parallel evolution; Convergent evolution; Homoplasy

"It is surprising how few special concepts one requires to understand development. Fate maps, asymmetric division, induction, competence, positional information, determination, and lateral inhibition will adequately cover most systems." Lewis Wolpert, 1994

Introduction

In 1994, Lewis Wolpert noted that many developmental systems could be described with a relatively small number of concepts: fate maps, asymmetric division, induction, competence, positional information, determination, and lateral inhibition (Wolpert, 1994). These seven concepts while quite broad are not exhaustive. In particular, additional molecular concepts such as genomics, redundancy, and genetic networks have come into more prominence in recent years. All these concepts were useful to guide observation and experimentation in traditionally amenable model systems during the birth and adolescence of developmental biology. As a result, over the past two to three decades, experimentation guided by these concepts has demonstrated that all organisms share many of the same molecular building blocks and many of the same regulatory genetic pathways (Gerhart and Kirschner, 1997; Hall, 1998; Raff, 1996; Wilkins, 2002). Yet we still do not understand how the various organisms use these molecules and pathways to assume all the forms we know today and this is the task of the field of evolutionary developmental biology. Evolutionary developmental biology at its heart is comparative biology, comparing the development of one organism to another and comparing the genes involved and gene functions to understand what makes one organism different from another. Like developmental biology itself, comparative developmental biology relies upon observation, description, and experimentation. Now that functional data is available

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for many genes in model organisms, whole genome sequences are available for perusal, and molecular tools have been developed that can cross species boundaries, are these concepts sufficient to describe changes in development among related organisms?

In this review, for each of these 10 concepts, we will provide a brief definition of the concept, discuss one example of a comparative developmental system where the concept has guided observations on the advent of a developmental novelty, discuss the experimental data, and place the system and results in an evolutionary context. Subsequently, we will see if any trends are to be found and will discuss if these concepts are as sufficient for describing the evolution of developmental mechanisms as they have been for understanding development itself. It should be noted that these descriptive concepts are not discrete and have conceptual overlaps. Also, many developmental events involve multiple steps, some of which may be described by one or more of these concepts. We concentrate on recent observations and areas of active study; we regret we cannot include all the work of interest being done.

Fate maps: nematodes and gonad morphology

A fate map traces the origin of a cell from founder cells or from a cytoplasmic domain in the zygote

The completed *C. elegans* cell lineage is in essence an extreme example of a fate map (Kimble and Hirsh, 1979; Sulston and Horvitz, 1977; Sulston et al., 1983). Comparison of the fate maps of different nematode species can identify changes that have occurred during evolution. Nematodes exhibit a diverse range of gonad morphologies (Malakhov, 1994). First, within a species, the gonad exhibits sexual dimorphism. Second, among species the morphology of the gonad varies in many ways including the number of gonad arms. The *C. elegans* cell lineage provides an explanation for gonad sexual dimorphism and the basis for a model for gonadogenesis (Fig. 1A and B).

C. elegans has two sexes: hermaphrodites, females that can make some sperm, and males. The hermaphrodite gonad is didelphic, consisting of two somatic rotationally symmetrical reflexed tubes that encase the germ line and unlaid embryos. The hermaphrodite gonad arms empty into the uterus, where embryos are stored while waiting to be expelled through the vulva, the nematode egg-laying organ. The ovaries, or arms of the gonad are patterned along a proximal-distal axis with respect to the vulva, the vulva is proximal (Fig. 1A, note adult diagram). The male gonad is monodelphic, consisting of a single gonadal arm that connects to the male tail (at the cloaca), which is specialised for mating; the somatic gonad and germ line are patterned proximal to distal with respect to the cloaca (Fig. 1B, note adult diagram).

The hermaphrodite and male gonad develop from an apparently identical symmetric gonadal primordium; however, an early cell migration establishes the asymmetrical male structure. In both sexes, the gonad starts from the same set of four cells: Z1 and Z4, the precursors of the somatic gonad arms, and Z2 and Z3, the primordial germ cells (Fig. 1A and B). In hermaphrodites the symmetry established in the gonadal primordium is maintained throughout development of the organ (Fig. 1A). In both hermaphrodites and males, the initial Z1 and Z4 division is symmetrical. In hermaphrodites, the progeny of Z1 and Z4 continue to divide in a symmetrical fashion. A single distal tip cell (DTC) is born from the early divisions of each of the somatic precursors. In hermaphrodites the DTCs have two functions; they act as leader cells for the extension of the gonadal arms and signal the germ line to maintain a mitotic germ cell population (Austin and Kimble, 1987, 1989; Crittenden et al., 1994; Henderson et al., 1994; Kimble and White, 1981; Yochem and Greenwald, 1989). Upon ablation of the DTCs, arms do not elongate and all germ cells exit mitosis, enter meiosis, and differentiate as gametes. In contrast, in males the progeny of Z1 and Z4 rearrange and break the symmetry in the developing gonad (Fig. 1B); ultimately, the two DTCs are found at the posterior and the remaining somatic cells at the anterior where a novel male-specific organiser, the linker cell (LC), serves as the leading cell for gonadal arm migration (Kimble and Hirsh, 1979).

The gonad becomes patterned upon formation of the somatic primordium (SP). Z1 and Z4 give rise to the founder cells of all adult gonad substructures, i.e., sheath, spermatheca, and uterus in hermaphrodites and seminal vesicle and vas deferens in males. These founder cells undergo a morphogenetic event that prepatterns the adult gonad. In hermaphrodites the somatic gonadal founder cells (except the DTCs) migrate centrally and coalesce to form the SP (Fig. 1A). In males the somatic gonadal cells cluster anteriorly, forming the male SP (Fig. 1B). Thus, the position of the somatic progenitor cells Z1 and Z4, the pattern of division of Z1 and Z4, and the migration of the resulting daughter cells gave an instant explanation for the difference in morphology between the hermaphrodite gonad and the male gonad.

The female/hermaphrodite gonad of many nematodes is composed of a single anterior reflexed arm (Fig. 1C and D, note adult diagram). Like *C. elegans* hermaphrodites, the lineages of these gonads usually start from four founder cells, two somatic cells and two germ line cells arranged in a rotationally symmetrical pattern. Unlike the somatic gonad founder cells of *C. elegans* hermaphrodites, Z1 and Z4 in these animals exhibit a number of differences (Felix and Sternberg, 1996; Sternberg and Horvitz, 1981). First, the synchrony of cell division may be broken between Z1 and Z4. For example, in *Panagrellus redivivus* (Fig. 1C, lineage), *Panagrolalaimus* sp. PS1579, and *Mesorhabditis* sp. PS1179 (Fig. 1D, lineage) Z4 divisions are retarded in comparison to those of Z1. Second, the progeny of Z4 often take on new fates. For example, the lineage that in *C.*

elegans gives rise to the posterior DTC in *C. elegans* undergoes programmed cell death in almost all of these species (Fig. 1C and D) (Felix and Sternberg, 1996; Sternberg and Horvitz, 1981). Thus, the premature death of the posterior DTC results in an enormous morphological novelty, a monodelphic gonad. This cellular mechanism differs from the one seen in the males of *C. elegans* and other nematodes. Why is the already existing cassette that results in a single gonad arm in males not coopted to produce a one-armed female gonad? A likely reason is that the program resulting in a one-armed morphology is also integrated into cell differentiation and tissue specification. As a result, this program may be developmentally constrained due to the necessity to alter pleiotropic developmental pathways.

Monodelphic hermaphrodite/female gonads may have evolved more than once in nematodes (Baldwin et al., 1997; Blaxter et al., 1998); for example, the monodelphic gonad lineages described for *Panagrellus* and *Mesorhabditis* are very likely to be independent events, and yet these morphological changes coopt the same pathway, programmed cell death (Felix and Sternberg, 1996; Sternberg and Horvitz, 1981). Thus, the nature of the molecular changes in the genomes of these lineages is an intriguing question; will they show any trends, i.e., are the same developmental pathways, maybe even the same genes, required to cause programmed cell death of the DTCs?

Asymmetric division: the origin of multicellularity in *Volvox*

Asymmetric cell divisions are cell division in which the daughter cells obtain different fates from one another, either by segregation of some cytoplasmic determinants or through signalling

Multicellularity likely evolved independently in the ancestors of the major eukaryotic groups, i.e., animals, plants, and fungi (Devereux et al., 1990; Sogin, 1991; Stechmann and Cavalier-Smith, 2002; Wainright et al., 1993). Unfortunately, little evidence remains of these ancient events. The advent of multicellularity likely required the evolution of several novelties, among them the generation of distinct codependent cell types, i.e., an asymmetric division resulting in two different daughter cell types. The green algae *Volvox* offers a unique opportunity to study the evolution of multicellularity and asymmetric divisions for several reason (Green and Kirk, 1981, 1982; Kirk, 1998). First, the simplicity of the system, the genus *Volvox* comprises a group of multicellular green algae composed of only two cell types: somatic cells specialized for motility (2000–4000 somatic cells in *Volvox carteri*) and gonidia, or asexual germline cells, specialized for reproduction, about 16 gonidia in *V. carteri* (Fig. 2A). These cell types are arranged in a specific manner: a single layer of flagellated somatic cells in a sphere that contains developing gonidia. Eventually, the somatic cells undergo programmed cell death releasing the progeny. Second, the genus *Chlamydomonas* is comprised of unicellular green algae closely related to *Volvox* that presumably exhibit the basal phenotype to provide a comparison for putative causal differences. Third, phylogenetic analysis suggests muticellularity arose relatively recently in the *Volvox* genus from a *Chlamydomonas*-like ancestor (Fig. 2D); thus, morphologically important changes are less likely to be clouded by background change that occurs over time; *V. carteri* and *Chlamydomonas reinhardtii* likely diverged from a common ancestor 50–75 million years ago (Rausch et al., 1989).

Gonidia result from asymmetric cell divisions (Green and Kirk, 1981, 1982; Kirk, 1998; Starr, 1970). Each gonidium is approximately 1000 times the size of a somatic cell in an adult organism. Each gonidium divides to produce all the cells, somatic and germ line, of the new organism. The initial divisions are symmetric up to the 32-cell stage and form a ball of cells. At the next division, the 16 cells in the anterior hemisphere, anterior is defined by the point of contact with overlying somatic cells, divide asymmetrically, each producing one large cell and one small cell. Each large cell gives rise to a single gonidium and divides asymmetrically two more times. Each small cell continues to divide symmetrically until they complete 11–12 rounds of division. Several lines of evidence suggest that it is the size of the cell that determines whether it differentiates as a gonidium or as a somatic cell. Large cells become gonidia; small cells differentiate as somatic cells. Any perturbations that interfere with cell division and result in larger cells give rise to excess gonidia differentiation (Fig. 2B) (Kirk et al., 1993); likewise, genetic perturbations resulting in excess divisions result in no gonidia differentiation (Fig. 2C) (Callahan and Huskey, 1980; Huskey et al., 1979; Kirk et al., 1991; Starr, 1970).

In *V. carteri*, three classes of mutations have been identified that affect somatic versus germline determination (Kirk, 1997): somatic regenerator mutations (*reg* genes), late gonidia mutations (*lag* genes), and gonidia-less mutations (*gls* genes). Representative genes from two of these classes have recently been cloned. In *reg* mutations, somatic cells fail to undergo cell death and redifferentiate as fully functional gonidia. All *reg* mutations map to a single locus, the *regA* gene (Huskey and Griffin, 1979; Kirk et al., 1987). The *regA* gene encodes a somatic cell-specific putative transcriptional repressor (Kirk et al., 1999). Potential targets for RegA regulation consist primarily of chloroplast-specific proteins (Choi et al., 1996; Meissner et al., 1999; Tam and Kirk, 1991a, 1991b). As *Volvox* is an obligatory autotroph, it has been hypothesized that *regA* prevents germline differentiation in somatic cells by restricting the number of functional chloroplast and thus inhibiting cell growth. In contrast, in Lag mutants gonidia differentiate as somatic cells and develop fully functional flagella. Later they re-

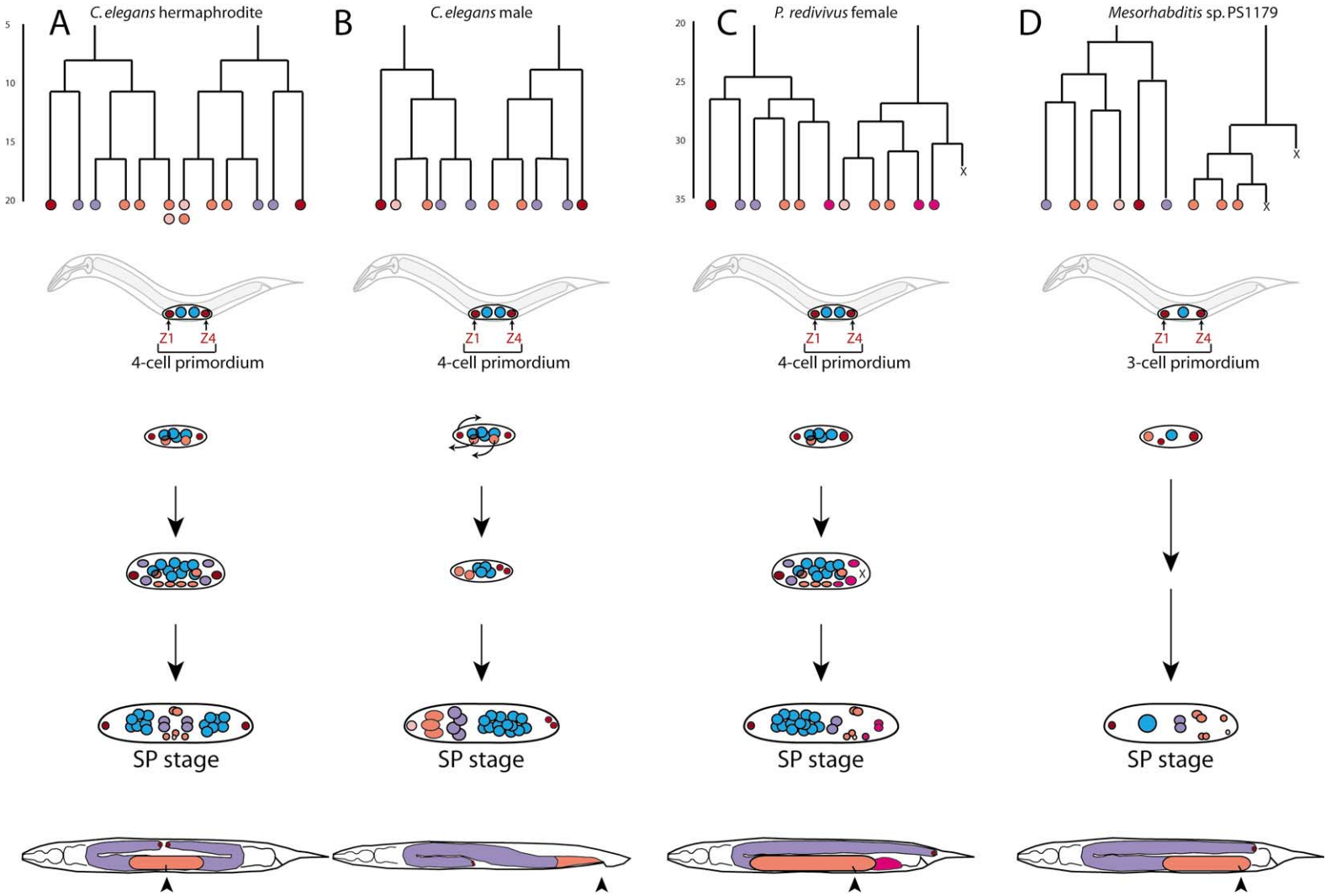


Fig. 1. Didelphic and monodelphic nematode gonads. Cell linage and gonad development in: *C. elegans* hemaphrodites (A), *C. elegans* males (B), *Panagrellus redivivus* females (C), and *Mesorhabditis* sp. 1179 females (D). Lineages: Line length represent the relative timing of divisions; an exact time scale is not available for *Mesorhabditis* sp. PS1179. Terminal Xs at the linage base represent cell deaths; terminal colours represent the predominant fate of the progeny of those cells: Dark red DTCs (distal tip cells): Purple, sheath/spermatheca in hermaphrodites/females and seminal vesicle in males; Red, uterus in hermaphrodites/females and vas deferens in males; Light pink, anchor cell in hermaphrodite/females and the linker cell in males; Dark pink, posterior pouch in *P. redivivus* females. Diagrams of gonad development: Diagrams of the initial somatic divisions and the somatic gonad primordium (SP) stage are shown for all animals; intermediate stages shown vary between animals. Small coloured circles represent the relative position of nuclei. Colours in the lineages correlate to the colours of individual nuclei at the SP stage. Arrows represent migrations in the *C. elegans* male gonad to break the symmetry of the gonad. *Mesorahditis* starts with a three-cell primordium as a single germline precursor is present. Arrowheads represent the position of the vulva in hermaphrodites/females and the cloaca. The position of the vulva in *P. redivivus* females is ~70% down the length of the body; the position of the vulva in *Mesorhabditis* is ~80% down the length of the body.

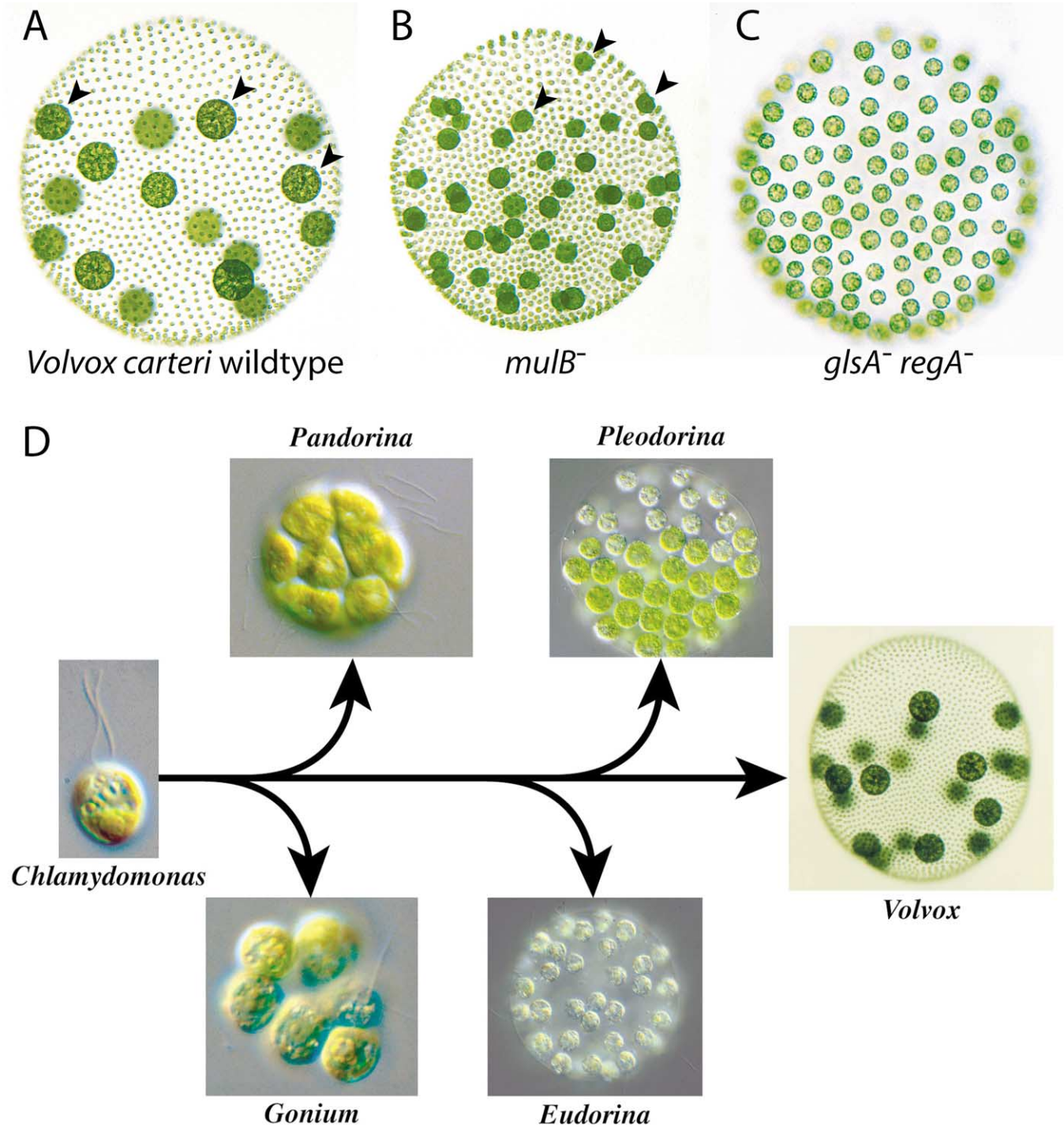


Fig. 2. Asymmetric divisions and the evolution of multicellularity in *Volvox carteri*. (A) A wildtype spheroid. (B) A Mul, multiple gonidia, spheroid. A *mulB* mutation results in a one-cycle delay in the occurrence of asymmetric divisions. As a result, there are more cells in the spheroid at the time of the asymmetric divisions and more cells undergo asymmetric divisions. This results in an increased number of large cells and a subsequent increase in the number of gonidia. (A and B) Arrowheads indicate representative gonidia. Smaller cells are somatic cells. (C) A GlsA RegA spheroid. A *glsA* mutation results in no asymmetric divisions. As a result there are no cells large enough to differentiate into gonidia and all cells differentiate as somatic cells. As GlsA mutants are sterile, they must be kept as a GlsA RegA double. In RegA mutants somatic cells redifferentiate as gonidia. (D) A traditional representation of related Volvocine algae showing a "progression" from a single cell form like *Chlamydomonas*, to colonial forms, to the multicellular *Volvox*. Organisms are shown left to right with progressively decreasing magnification. Single biflagellated cells, i.e., all cells excluding gonidia, range from 5 to 10 (μm in diameter).

differentiate as gonidia. *lag* mutations map to four loci (Kirk, 1998). While no *lag* genes have been cloned to date, it is assumed that they act in gonidia to suppress somatic differentiation. Last, Gls mutants never divide asymmetrically and all cells differentiate as somatic cells (Fig. 2C) (Green and Kirk, 1981, 1982; Kirk, 1998; Starr, 1970). As

Gls mutants are sterile on their own, they must be maintained in a RegA mutant background. Additionally, Gls mutant strains have been difficult to cross into each other; thus, complementation tests to define the number of loci have yet to be reported. The *glsA* gene has been cloned and encodes a chaperone protein with a functional J-domain and likely acts with an Hsp70-like partner (Miller and Kirk, 1999). GlsA is associated with the mitotic spindle and its expression peaks in asymmetrically dividing embryos, which is consistent with a proposed role in controlling the position of the spindle during asymmetric cell division.

It has been proposed, based upon the life cycle of its unicellular relatives, such as *C. reinhardtii*, that *V. carteri* evolved from a single cell ancestor with a biphasic life history (Kirk, 1999). Initially, this single-cell ancestor existed in a somatic stage complete with a flagellum to allow it to regulate its position with the water's surface to maximize access to sunlight. Following a light-dependent growth phase, this ancestor may have then redifferentiate into a reproductive stage. Following this hypothesis, and based upon the three classes of mutations discussed, it is proposed that the *V. carteri* phylogenetic lineage evolved a set of repressors, one set used in somatic cells to repress the reproductive cycle and one set used in germ cells to repress the earlier growth life cycle. Consequently, these genes are likely to have played a central role in the evolution of asymmetric divisions and the generation of two discrete cell types, and thus in the evolution of multicellularity in *V. carteri*. The cloning of these genes open the potential to determine the function of their homologues in related single-celled relatives, and in related algae with clonal morphologies intermediate between *Volvox* and *Chlamydomonas*.

The Volvocines comprise a group containing *Volvox, Pleodorina, Eudorina, Pandorina,* and *Gonium* (Kirk, 1998). These other taxa have been hypothesized to represent intermediate stages of multicellular evolution between *Chlamydomonas* and *Volvox* (Fig. 2D). Recent molecular phylogenetic analysis indicates that many of these volvocine lineages are polyphyletic; as a result it appears that multicellularity in this phylogenetic lineage has evolved independently many times in the recent past (Coleman, 1999; Larson et al., 1992; Nozaki and Ito, 1994; Nozaki et al., 1995, 1997, 2000). Additionally, the phylogenetic data also suggest that some of these phylogenetic lineages may have changed back to simpler colonial forms from more complex forms (Nozaki et al., 2000). In support of this, mutational analysis of more complex forms shows that only one or a few changes are required for a reversion to more primitive forms (Tam and Kirk, 1991b; Vande Berg and Starr, 1971). As with many of the other areas of investigation we are touching upon in this review, it will be interesting to see if in these separate yet closely related lineages, the nature of the changes found shows any trends.

Induction: eye loss in cavefish

Induction is a process in which a cell or tissue signals to another cell or tissue to effect its developmental fate

The potential for fish to become geographically isolated within known geological time frames makes them an excellent system for investigation of population genetics, evolution, and adaptation. Two recent studies use endogenous wild populations to identify loci involved in the development of specific morphological features, one using cavefish (Yamamoto and Jeffery, 2000) and one using stickleback fish (Peichel et al., 2001); in this section we review the cavefish study where changes in induction have been shown to result in novel eye phenotypes. *Astyanax mexicanus* is endogenous to parts of the southwest United States and Mexico and populations of this species have evolved to live in cave ecosystems. Hypogean, or subterranean, populations of *A. mexicanus*, exhibit a set of adaptations common to animals living in the dark (Jeffery, 2001; Mitchell et al., 1977). One of these is the loss of eyes (compare Fig. 3A and B). Eye development in cave-dwelling fish proceeds along a similar path as its epigean, or surface dwelling, counterpart. During eye development, an optic vesicle forms and a thickening of the surface ectoderm forms the lens placode that buds into the optic cup. The first point of divergence occurs during lens differentiation: The fiber cells of the lens, which normally elongate and begin to express crystalline proteins, do not terminally differentiate and a large amount of apoptosis is observed (Jeffery and Martasian, 1998; Yamamoto and Jeffery, 2000). Perhaps due to the degeneration of the lens, the iris, cornea, and pupil do not develop. The retina, which initially forms properly, never differentiates photoreceptor cells and exhibits apoptosis and degenerates. Ultimately, the degenerate eye sinks into its orbit and is covered by a flap of skin.

To investigate the role of the lens in eye formation, Yamamoto and Jeffery (2000) performed lens transplantation experiments. A hypogean lens vesicle was placed in an epigean optic cup in which the lens vesicle had been removed. The hypogean lens underwent apoptosis and degeneration, and like the eye of a cavefish, the surface-dwelling fish eye with the transplanted lens did not develop an iris, cornea, or anterior chamber (Fig. 3D). This experiment suggested that apoptosis in the lens is lens autonomous. In addition, the retina appeared less organised and smaller than the control surface fish eye as well, though it did differentiate appropriately. In the reciprocal experiment, an epigean lens vesicle was placed in a hypogean optic cup in which the endogenous lens vesicle was dissected. The transplanted cavefish eye developed normally with an iris, a cornea, and an anterior chamber (Fig. 3C). The retina exhibited proper organisation and differentiated appropriate cell types such as rod cells in the rescued eye in comparison to the control cavefish eye. Together, these results indicate that the lens vesicle of surface fish is capable of inducing the optic cup of both epigean and hypogean fish to promote eye development, while the cavefish lens vesicle has lost this ability. These

experiments open new opportunities to study the underlying changes in induction: Is the degeneration of the lens the causal difference between hypogean and epigean populations and would the inhibition of cell death in the lens restore eye function? It is interesting to note that changes in the control of cell-death is a recurring theme in many of the developmental systems compared, whether the death of an organising cell in nematode gonads, the death of somatic cells in *Volvox*, or the death of the lens in cavefish to remove an inductive signal.

Candidate molecules involved in vertebrate eye development are under investigation for playing roles in eye loss (Jeffery et al., 2000; Strickler et al., 2002). Among the genes that have been looked at, changes in *Pax6* expression may have played a role in cavefish eye loss (Strickler et al., 2001). *Pax6* encodes a paired-class homeodomain transcription factor expressed during teleost eye development. *Pax6* homologues have been shown to be important in eye development from flies to mice (Kumar, 2001; Pichaud and Desplan, 2002). Analysis of *Pax6* mRNA expression in the cavefish eye compared to the surface fish eye shows a decrease in the overall domain of expression. In particular, there is a reduction in *Pax6* expression in the eye primordia and, earlier in development, a gap of expression at the anterior margin of the neural plate. Additionally, Pax6 protein expression is also reduced during cavefish lens development in comparison to surface fish. It was hypothesized that the gap in *Pax6* expression may be the result of increased midline signalling by hedgehog.

Recent phylogenetic analysis indicates that over the past million years, there may have been multiple isolation incidents resulting in discrete populations of hypogean *A. mexicanus* (Dowling et al., 2002). Thus, eye loss in these different cavefish may have resulted independently. Preliminarily, the lens may have a similar role in eye loss in some of these other strains as a high degree of apoptosis in the degenerating lens is also observed (Jeffery and Martasian, 1998). Does eye loss result from similar changes at similar levels in the same pathway or do they involve different genes at every level of a pathway or even entirely independent genetic pathways? Like the advent of monodelphic gonads in nematodes and multicellularity in *Volvox*, eye loss offers the opportunity to study the robustness of genetic pathways.

Developmental genomics: QTL analysis of skeletal morphology among threespine stickleback fish species

Developmental genomics is the creation and use of tools and techniques to study differences in the genomes and the biological functions of individual genes among related organisms

The skeletal architecture of vertebrates is widely divergent, yet the basis for change in gross skeletal morphology remains almost entirely unknown. Once again, studies of isolated populations of fish may bring insights into important issues in vertebrate evolution. *Gasterosteus aculeatus*, the marine threespine stickleback fish, has undergone recent rapid speciation events (Bell and Foster, 1994). During the past 15,000 years, as glaciers retreated, populations of marine threespine stickleback fish colonised newly formed lakes and rivers along the northwest coast of North America. These isolated populations rapidly diverged and experienced both allopatric speciation (speciation due to geographical isolation) and sympatric speciation (speciation due to ecological or behavioural isolation). In particular, in many lakes discrete benthic and limnetic stickleback species evolved. Sympatric species exhibit adaptive changes specific for their new niches; among these adaptations are differences in skeletal morphology. The marine threespine stickleback fish has a number of easily observable skeletal features, among them three dorsal spines, two pelvic spines, and a series of lateral armour-like bone plates. Benthic species live near the shore and have a reduction in body armour: dorsal spines, pelvic spines, and lateral bony plates are either reduced or absent. These changes may be a response to increased predation by insects along the shore where a reduction in "handholds" maybe advantageous (Reimchen, 1980, 1983). In contrast limnetic species retain much of the body armour of their ancestral marine relative; life in the open-water may favour protective armaments because of increased predation by larger vertebrates (Hagen and Gilbertson, 1972; Moodie, 1972; Reimchen, 1980).

A recent study has generated a genetic map for the threespine stickleback fish and performed quantitative trait loci (QTL) analysis to map putative genetic changes that have resulted in different skeletal morphologies between a benthic and limnetic species pair (Peichel et al., 2001). Animals do not frequently mate across species boundaries in the wild (Hatfield and Schluter, 1999; Nagel and Schluter, 1998; Ridgway and McPhail, 1984; Rundle et al., 2000; Vamosi and Schluter, 1999); however, in vitro fertilization is easily accomplished between species in a laboratory setting (McPhail, 1994). To generate a linkage map, a series of molecular genetic markers, CA dinucleotide-based microsatellite sequences, that were polymorphic between the benthic and limnetic stickleback species, were identified. Benthic/limnetic hybrid animals were generated and F1 hybrid males were backcrossed to benthic females. The F2 were scored for benthic and limnetic microsatellite markers to establish linkage between markers; 227 markers were placed into 26 linkage groups. Following the generation of the map, individual F2 hybrid animals were scored for various morphologies including the number and size of spines and the amount of body armour. Individual phenotypes were correlated with the presence or absence of parental genetic markers. For many traits, loci were identified that are likely to be important for the generation of specific skeletal structures.

Genetic maps and QTL analysis have provided a highly promising beginning for the exploration of the events that have resulted in different morphologies among stickleback fish species. Thus, the use of genomic tools in evolutionary developmental biology opens up new avenues of investiga-

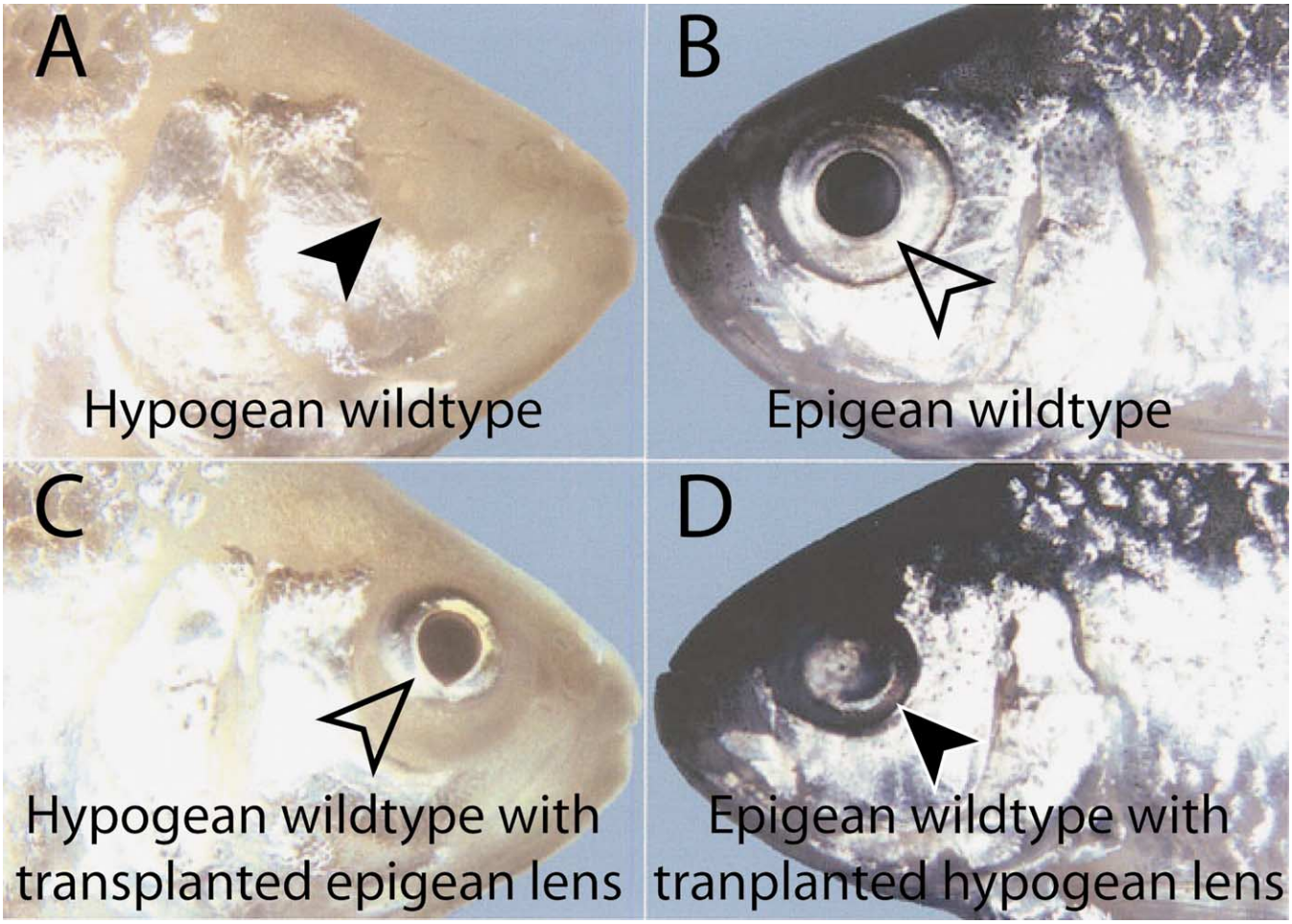


Fig. 3. Lens transplantation experiments in *Astyanax mexicanus*. Two fish with transplanted lenses. Open arrowheads indicate a well-developed eye. Solid arrowheads indicate the absence of an eye. (A) The control side of a cavefish. (B) The control side of a surface fish. (C) The transplant side of a cavefish. The endogenous lens has been removed and replaced with a lens from a surface fish. Relatively normal eye development occurs. (D) The transplant side of a surface fish. The endogenous lens has been replace with a cavefish lens. No eye is present.

tion. When the set of genomic techniques is completed so that an integrated genetic and physical map is accessible and transgenic and gene knockdown techniques are available, the cloning of identified quantitative trait loci is possible.

In addition, more in-depth studies using QTL analysis offer the potential to investigate fundamental issues in evolutionary biology, including the robustness of developmental processes and convergent evolution. Stickleback species have recently diverged over a very short time period. In some of the systems discussed, background variation in wild isolates and redundant mechanisms act to reduce an organism's dependence upon the function of individual genes within a discrete process. For the different stickleback species, as time progresses, a scenario could be envisioned where additional genes may come to assume redundant roles and enough background variation may mask or reduce the effect of individual genes on a specific trait. This opens the potential to address questions about robustness of traits and the generation of genetic variation and redundant mechanisms. As species diverge, do the genes initially responsible for a new morphology retain the same degree of importance for maintaining that trait? How quickly does a trait become robust in wild populations due to the generation of variation and redundancy? These questions also beg the question of the effectiveness of QTL analysis for more distantly diverged species, assuming hybrid animals can be generated. Additionally by looking at geographically isolated sympatric benthic and limnetic species pairs, one can begin to address questions about the convergent evolution of traits. Does QTL analysis of discrete sympatric pairs result in the identification of the same putative loci?

Competence: changes in nematode vulva equivalence groups and the origin of posterior vulvae

Competence is the ability of a cell or tissue to respond to an external signal, often from an adjacent cell or tissue

Among nematodes, the position of the vulva is highly variable (Fig. 1A, B, and C) (Sommer and Sternberg, 1994).

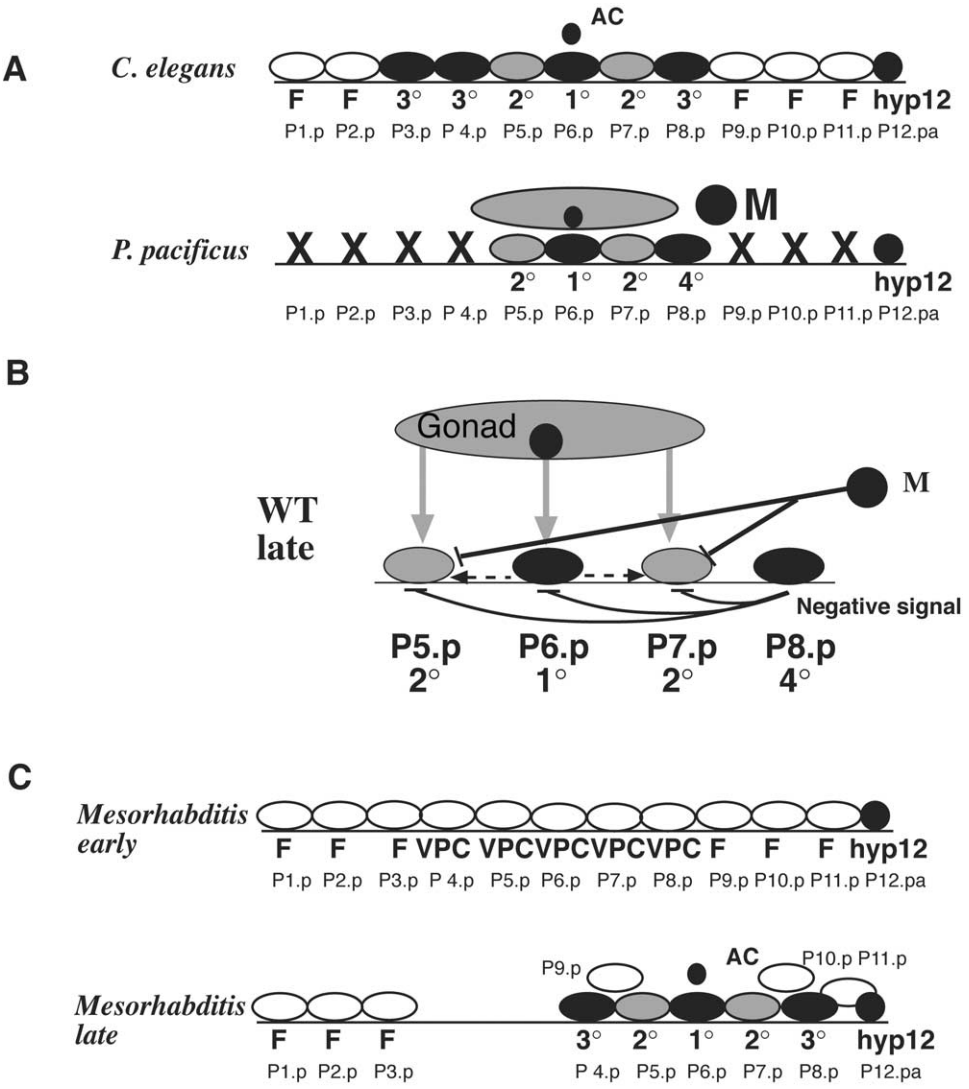


Fig. 4. Changes in nematode vulva development across phyla. (A) A summary of changes in vulva induction between *C. elegans* and *P. pacificus: i.* The size of the equivalence group changes during evolution. In both animals the 12 ventral hypodermal precursor cells are equally spaced between the pharynx and the rectum. In *C. elegans* P(1, 2, 9–11).p fuse with the hypodermis prior to induction of the gonad. In *P. pacificus* P(1–4, 9–11).p undergo programmed cell death prior to induction of the vulva. *ii.* The inductive signal changes. In *C. elegans* the anchor cell (AC) signals over a short time span, whereas in *P. pacificus* the gonad signals over an extended period. In both animals P6.p forms the centre of the vulva and has a 1° fate and P(5, 7).p form the outside of the vulva and have a 2° fate. The remaining Pn.p cells remain epidermal. *iii.* There is a new inhibitory signalling centre in *P. pacificus*, P8.p. (B) A model for signalling in *P. pacificus.* In the absence of gonad signalling P8.p inhibits ectopic vulva formation in P(5–7).p. The gonad signals to the P(5–7).p to induce the vulva over an extended period of time. The induced P6.p signals to P(5, 7).p to promote 2° fates. P8.p also laterally inhibits 1° fates in P(5, 7).p, mediated by mesoblast M. (C) Formation of the posterior pharynx from central Pn.p cells in *Mesorhabditis* sp. 1179. The central Pn.p cells migrate to the posterior where the migrating AC meets the developing precursor cells to establish the connection between the uterus and the vulva. The induction of the vulva in *Mesorhabditis* is AC independent.

The vulva is composed of rings of cells that attach the uterus to the epidermis of the animal (White, 1988). In many nematodes, such as *C. elegans*, the vulva is located in the middle of the body along the anterior/posterior axis. In other nematodes, mainly nematodes with monodelphic female/hermaphrodite gonads, the vulva is found near the posterior of the animal (Sommer and Sternberg, 1994). Phylogenetic analysis indicates that the former is likely ancestral and the second a derived feature that has evolved independently in most of these species (Baldwin et al., 1997; Blaxter et al., 1998).

Organogenesis of the *C. elegans* vulva is one of the best understood processes in animal developmental biology (Wang and Sternberg, 2001). The *C. elegans* vulva is formed by the progeny of six of twelve hypodermal precursor cells P(3–8).p that lie in a line along the ventral cord of the animal (Fig. 4A) (Sulston and Horvitz, 1977; Sulston and White, 1980). The remaining hypodermal cells fuse into

a syncytial cell earlier during development. The developing gonad lies along the body of the developing larva and contacts the progenitors of the vulva. A single somatic gonadal cell, the anchor cell (AC), signals to these epidermal cells to induce the vulva via a conserved EGF/Ras/MAPK signal transduction pathway (Sternberg et al., 1994). This signal is received in a graded fashion (Katz et al., 1995). P6.p is immediately adjacent to the AC and is induced to form the central part of the vulva and has a 1° fate (Fig. 4A). The adjacent cells P5.p and P7.p form the outer part of the vulva and have 2° fates. Upon induction P6.p sends a second signal, involving the Notch-like molecule LIN-12, to P5.p and P7.p to induce 2° cell fates (Koga and Ohshima, 1995; Simske and Kim, 1995). The remaining cells assume a 3° fate and fuse with the epidermis. While the vulva is formed from P(5–7).p, all six vulval precursor cells are competent to receive the signal; cell ablation of P(5–7).p results in the ablated cells being replaced by their closest neighbours (Sulston and White, 1980).

Despite a similar morphology for the vulva, many nematodes display a surprising degree of change at the molecular level in the way the vulva is generated. First, many nematodes have a very different induction mechanism. The inductive signal can be gonad dependent or independent, occur in multiple stages, require a continuous signal, and/or can be sent by a single cell or group of cells (Fig. 4A and B) (Sommer, 2000b). Second, the size and composition of the equivalence group can change. The vulva equivalence group of *Pristionchus pacificus* consists of P(5–8).p (Sommer and Sternberg, 1996). Nematodes belonging to the Suborder Cephalobina, even more distant from *C. elegans*, can have expanded equivalence groups, i.e., *Panagrellus redivivus* P(3–9).p (Felix et al., 2000; Sternberg and Horvitz, 1982). Worthy of note, while cell fusion plays a role in limiting the size of the equivalence group in *C. elegans*, in many other nematodes programmed cell death is used to limit the equivalence group (Fig. 4A). Last, new organising centres can evolve; in *P. pacificus*, P8.p limits P(5,7).p to adopting 2° fates in response to the gonadal signal upon ablation of P6.p (Fig. 4B) (Jungblut and Sommer, 2000). Ablation of P6.p and P8.p allows either P5.p or P7.p to adopt a 1° fate. Thus, an inhibitory signal has evolved to prevent a cell fate, somewhat reminiscent of what has been hypothesised for *Pax6* expression and eye loss in cavefish.

Given the mutability of vulva induction, it might be expected that nematodes with a posterior vulva would have a novel posterior equivalence group that would be induced at the posterior of the animal. Yet in three nematodes studied, the posterior vulva is still formed from the central Pn.p cells, which subsequently migrate to the posterior (Fig. 4C) (Sommer and Sternberg, 1994). The somatic gonad cells that comprise the uterus extend toward the posterior as well. Eventually, the AC makes contact with the equivalence group to form the vulva, though an inductive signal from the anchor cell or gonad is not required for vulva induction in most of these nematodes (Sommer and Sternberg, 1994). A preference for the de novo generation of the migration of the central Pn.p cells and the elongation of the somatic gonad may represent the presence of a developmental constraint. Theoretically, the use of posterior precursor cells to form a vulva could be accomplished in two ways. First, a vulva program could be induced in the posterior cells by the evolution of a hermaphrodite-specific signalling mechanism dependent upon a posterior *Hox* gene. *mab-5* is the *Hox* gene known to regulate the development of the posterior body region in hermaphrodites and males of *C. elegans* and *P. pacificus* (Jungblut et al., 2001; Jungblut and Sommer, 1998; Kenyon, 1986) and a *mab-5*-dependent signalling system induces the formation of the hook, a male-specific mating structure, from posterior epidermal cells (Maloof and Kenyon, 1998). A posterior vulva could theoretically be induced if in hermaphrodites this *mab-5*-dependent signalling could target a vulva developmental cassette instead of the male-specific hook. Second, a vulval program could be induced in the posterior by the evolution of a new domain of expression of the mid-body *Hox* gene *lin-39*, the gene that determines the vulval equivalence group in *C. elegans* and *P. pacificus* (Clark et al., 1993; Eizinger and Sommer, 1997; Wang et al., 1993). In *C. elegans* it has been shown that ectopic expression of *lin-39* in the posterior of *C. elegans* results in posterior vulva-like invaginations (Maloof and Kenyon, 1998). That none of these potential solutions have been used in nematodes strongly suggests that the pleiotropic nature of *Hox* gene function and the complex network of cell interactions may make these changes difficult to execute. Instead, it is "easier" to have the future vulva cells adopt an autonomous behaviour, cell migration, to form a vulva in the posterior region.

Genetic redundancy: the functions of *bicoid*, *hunchback*, and *orthodenticle* in establishing the anterior-posterior axis in insects

"Genetic redundancy means that two or more genes are performing the same function and that inactivation of one of those genes has little or no effect on phenotypes." (Nowak et al., 1997)

As has already been seen in nematode vulva development, one general property of essential developmental processes is that they are robust; that is, the process is often bolstered by background genetic variation and redundant developmental mechanisms to allow compensation for some changes in the gene network. As we discuss later in the conclusions, these backup mechanisms can allow for dramatic changes even in fundamental mechanisms. A striking example of this is the hypothesis for the evolution of *bicoid* (*bcd*) function in establishment of anterior positional information *Drosophila.*

bicoid plays a central role in specifying the anterior of the *Drosophila* embryo; as such, it is a member of the

anterior group genes, which act with the posterior group genes and the terminal group genes to establish the anterior-posterior axis (Rivera-Pomar and Jäckle, 1996; St. Johnston and Nusslein-Volhard, 1992). *Drosophila* embryos lacking *bcd* have broad defects in head and thoracic segments. *bcd* RNA is maternally supplied to the egg and localised to the anterior pole via elements in the mRNAs 3′ UTR. It is translated upon fertilisation to establish a gradient of Bicoid protein, high levels in the anterior and low levels in the posterior. *bcd* is located in the *Drosophila Hox* cluster and encodes a homeodomain transcription factor (Struhl et al., 1989). The Bcd protein has several unusual features: First, Bcd can bind both DNA and RNA (Rivera-Pomar et al., 1996). Second, Bcd has a lysine at position 50 of the homeodomain; the other members of the Drosophila *Hox* cluster all contain a glutamine at position 50. This lysine strongly affects the target binding specificity of Bcd (Treisman et al., 1989). Bcd acts to promote transcription of anterior genes, such as *hunchback* (*hb*), and to inhibit translation of posterior genes, such as the maternal ubiquitously expressed *caudal* (*cad*) mRNA. Establishment of Hb and Cad protein gradients, high Hb levels in the anterior and high Cad levels in the posterior, is essential to proper anterior-posterior patterning and specification of gap genes. Consistent with their roles as a gap gene and a maternal posterior gene, mutations in *hb* result in discrete segment defects (Lehmann and Nusslein-Volhard, 1987) and mutations in *cad* result in abdominal defects (Macdonald and Struhl, 1986; Rivera-Pomar et al., 1995).

Phylogenetic evidence suggests that *bcd* may be a new innovation in the anterior positional information gene network during the evolution of Dipterans. Despite repeated attempts, it has not been possible to clone *bcd* homologues outside of the Cyclorraphan flies (Stauber et al., 1999). Additionally, *bcd* is not present in the *Antennapedia* complex of the flour beetle *Tribolium castaneum* (Brown et al., 2002). This has caused speculation that *bcd* may have evolved late in the evolution of the Dipterans. Recent molecular phylogenies strongly suggest that *bcd* is a highly divergent Hox3 class gene that may have resulted from the duplication of a *zerknüllt* (*zen*)-like ancestor. Molecular phylogenies of *Hox* genes in the Cyclorrhaphan fly *Megaselia abdita* support the hypothesis that the *zen* and *bcd* orthologues are sister genes within the *Hox* family (Stauber et al., 1999). Additionally, *zen*-like genes have been cloned in non-Cyclorraphan flies more distantly related to *Drosophila*; these *zen*-like genes have an expression pattern analogous to the composite expression pattern of Drosophila *zen* and *bcd* (Stauber et al., 2002).

bcd does not appear to be as central to establishing anterior positional information as initially thought in *Drosophila*. Ectopic expression of Hb is able to partially rescue *bicoid* defects in thoracic segments (Wimmer et al., 2000). This is one indication that *hb* is actually the central player in anterior patterning and that a principal function of *bicoid* is to regulate *hb* and that *hb* and other anterior group genes are more directly involved in anterior patterning. In support of this, a recent study has demonstrated that the Bcd protein gradient is highly variable in terms of expression level at a given position with respect to the length of individual *Drosophila* embryos (Houchmandzadeh et al., 2002); thus, variation in *bcd* expression levels have little effect on patterning. In contrast, the Hb gradient is highly reproducible with respect to embryo length among *Drosophila* embryos. Furthermore, this invariance in *hb* expression levels is likely to be modulated by genes other than *bcd*. Third, *orthodenticle* (*otd*) and *hb* may partially substitute for *bcd* in anterior embryonic patterning in *Tribolium* (Schröder, 2003; Schulz and Tautz, 1995; Wolff et al., 1995). Otd is a homeodomain protein that functions in *Drosophila* as a zygotically expressed head gap gene (Cohen and Jurgens, 1991). *Tribolium* has at least two orthodenticle genes (Li et al., 1996). Only *Tribolium otd-1* is expressed in the anterior during early embryogenesis. Unlike *Drosophila otd*, *otd-1* is contributed to the *Tribolium* egg maternally. *otd-1* mRNA is initially ubiquitous in the early embryo but becomes subsequently localized to the anterior. Expression eventually becomes sequestered to a broad band in the prospective head region. Gene knockdown experiments of *otd-1* in *Tribolium* result in stronger anterior patterning defects than those observed for *otd* loss-of-function mutant *Drosophila* embryos. Double gene knockdowns in *Tribolium* with *otd-1* and *hb* result in severe anterior patterning defects analogous to *bcd* loss-of-function mutant *Drosophila* embryos. In comparison to *bcd*, *otd* is an ancient conserved patterning gene. Intriguingly, like Bcd, Otd has a lysine at position 50 of its homeodomain (Finkelstein et al., 1990).

Taking all data together, it has been proposed that *bcd* may have assumed the functions of more ancient patterning genes (Dearden and Akam, 1999; Schröder, 2003; Stauber et al., 1999, 2002). Initially, a *Hox* class 3 *zen*-like gene, expressed in extraembryonic membranes and early in embryogenesis, was duplicated during Dipteran evolution. The two genes evolved discrete functions, *zen* retained its expression and function in the extra-embryonic membranes and *bcd* retained expression in the early embryo where it was free to assume a role in anterior patterning. A subsequently mutation at position 50 allowed *bcd* to recognise and regulate *otd* targets, such as *hb*. *bcd* and *otd* became redundant. With *bcd* assuming its maternal embryonic functions, *otd* was no longer required for these functions and evolved into a head gap gene. Three counter arguments exist to this proposal. First, it may not be easy to clone or recognise *bcd* orthologues from phyla more distantly related to *Drosophila* because the *bcd* homeodomain has evolve quickly within the Cyclorraphan flies (Schröder and Sander, 1993). Second, *hb* and *cad* are expressed early in *Tribolium* embryos in a similar pattern to their *Drosophila* orthologues, high Hb in the anterior and high Cad in the posterior (Wolff et al., 1995). Expression constructs using *Tribolium hb* and *cad* regulatory sequences are appropriately regulated in *Drosophila* embryos (Wolff et al., 1998). This regulation

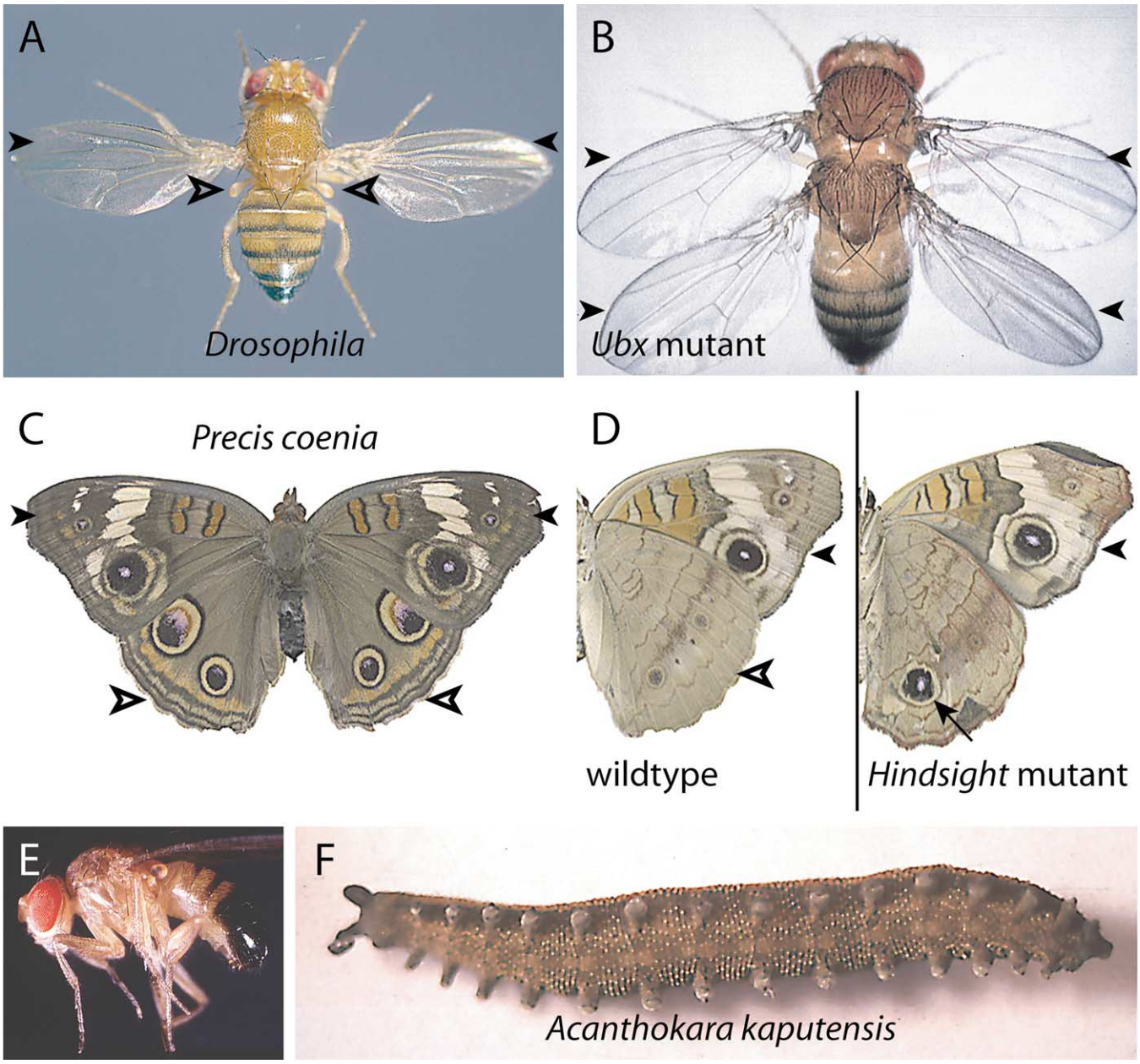
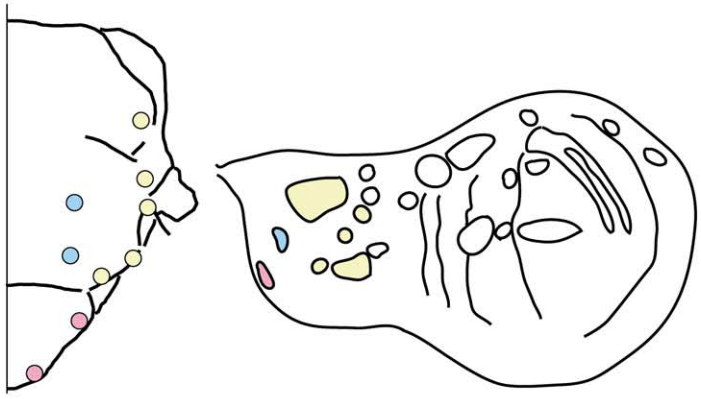


Fig. 5. Changes in appendage morphology in insects and related phyla. (A) *Drosophila* wild type. (B) *Ubx* mutant *Drosophila.* Solid arrowheads indicate wings; open arrowheads indicate halteres. (C) Dorsal view of *Precis coenia* wild type. (D) Ventral views of *P. coenia* wings: left, wild type; right, a *Hindsight* mutant. Hindsight mutants lack expression of Ubx in patches in the hindwing resulting in forewing expression patterns, indicated by arrow. Forewings indicated by solid arrowheads; hindwings indicated by open arrowheads. (E) Side view of wild-type *Drosophila* showing three pairs of legs coming off the thorax. (F) Ventral view of an onychophoran, *Acanthokara kaputensis*, showing pairs of appendages along the entire trunk.

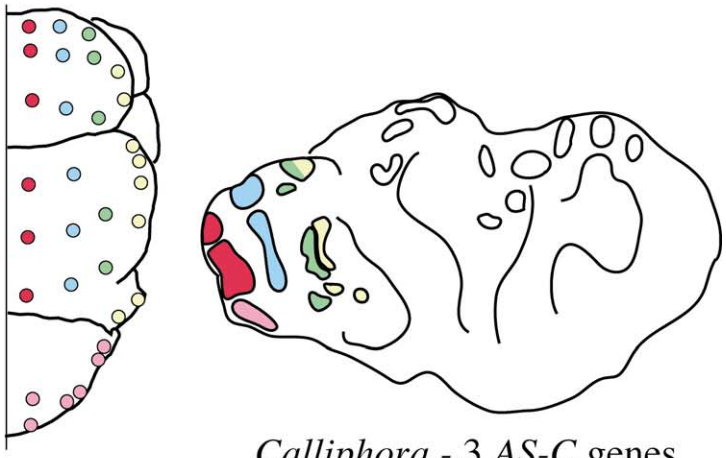
Fig. 6. *achete-scute* (*ac-sc*) expression and microchaete and macrochaete patterning. (A) *Drosophila* notum showing microchates and macrochates; diagrams of the right side of a *Drosophila* notum and the wing imaginal disk, showing the position of proneural clusters defined by domains of expression of *achete-scute* complex (*AS-C*) genes. Proneural clusters prefigure the site of macrochaete development. (B) Wild-type blowfly notum showing microchaetes and macrochaetes; diagrams of the right side of a blowfly notum showing the position of proneural clusters defined by domains of expression of *achete-scute* complex (*AS-C*) genes. Different colours represent domains established by discrete *cis*-regulatory elements in the AS-C in their respective species. (C) Left, a diagram of notum of a mosquito showing the position of macrochaetes, large black circles, and microchaetes, red dots; right, a digram of a mosquito notum showing the expression domain of an *Anopheles pannier*, *Agpnr*, and an *Anopheles achete-scute*, *AgASH*, homologue with respect to the future position of macrochaetes, which are shown as solid colored dots. *Agpnr* and *AgASH* have identical expression domains through development. In the fourth larval instar, they are first expressed in two stripes that run the length of the notum, light green; subsequently expression is limited to a triangle at the posterior of the notum, light red; ultimately expression is confined to a kidney shaped domain in the posterior.

A



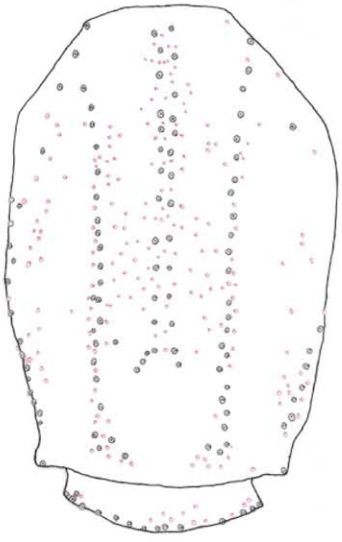
Drosophila - 4 *AS-C* genes

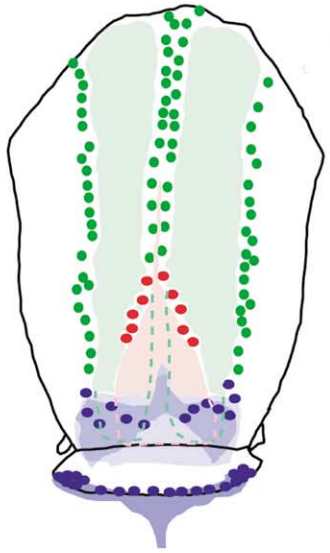
B



Calliphora - 3 *AS-C* genes

C





Anopheles - 2 *AS-C* genes

is *bcd* dependent, suggesting that a *bicoid*-like activity exists in *Tribolium*. Third, *otd-1* is not necessary for *hb* transcription in *Tribolium* and the *otd-1* homeodomain lacks specific amino acids that are required in *Drosophila* for translational regulation of *cad*. Indicating that *otd-1* likely does not assume the molecular functions of *bcd* in *Tribolium* (Schröder, 2003).

Positional information: ultrabithorax and changes in arthropod limbs

Positional information refers to an underlying informational field that cells sense and interpret according to their genetic background and developmental history, which provides the basis for pattern formation

Arthropods display some of the most divergent body plans of any animal phylum. While the final morphology of different arthropods is highly variable, they start from a similar anlage, an anterior-posterior linearly segmented body with appendages protruding from the segments. One of the most striking differences between arthropods is the number and the morphology of the limbs. *Hox* genes are conserved in all multicellular animals and universally used to establish positional information along the anterior-posterior body axis of all animals studied (Duboule, 1998; Gellon and McGinnis, 1998). Not surprisingly, the *Hox* genes also regulate limb formation in the arthropod segments (Averof, 2002; Browne and Patel, 2000; Gardner, 2001). Changes in the expression domains of *Hox* genes, changes in the developmental cassettes activated by *Hox* genes, and changes in the amino acid sequence of regulatory domains within Hox proteins have all been shown to affect whether an arthropod segment can bear a limb and the type of limb that is present on a segment. We will focus on changes in the *Hox* gene *Ultrabithorax*, *Ubx*, which have resulted in altered morphologies of insect wings and in restricting the segments that can bear limbs.

Drosophila, a dipteran, bears a pair of wings and a pair of halteres (Fig. 5A). Dipterans evolved from insects with four wings and the *Drosophila* halteres are homologous to a second pair of wings, which themselves are serially homologous to the first pair of wings. It has been shown that the expression of *Ubx* in the haltere but not the wing is responsible for the morphological differences between these appendages (Fig. 5B) (Weatherbee et al., 1998). Ubx inhibits wing formation to promote haltere development by selectively repressing Wingless (Wg) signal, Wg-activated genes, and Decapentaplegic (Dpp)-activated genes; Wg is a Wnt family member and Dpp is a TGF-β family member. Ubx appears to act through *cis*-acting enhancers to regulate these genes. For example, *vestigial* (*vg*) is expressed twice in wing development, once in the dorsal/ventral boundary of the disc and once in the growing wing pouch. In contrast, *vg* is only expressed once in the haltere disc at the dosal/ventral boundary, but is repressed in the wing pouch to suppress wing growth, through an Ubx-sensitive enhancer element (Weatherbee et al., 1998). Thus, changes in the domain of expression of *Ubx* have resulted in changes in morphology between homologous structures.

Butterflies (Lepidoptera) have two distinct sets of wings, a forewing and a hindwing (Fig. 5C). Like in *Drosophila*, *Ubx* is expressed in the developing hindwing, but not the forewing. Mutant butterflies of the species *Precis coenia* that lack *Ubx* expression in patches of the hindwing show forewing patterning in these patches (Fig. 5D); thus, like in the *Drosophila* haltere, *Ubx* is likely to play a role in the different morphologies (Weatherbee et al., 1999). However, in comparison to expression patterns in the *Drosophila* haltere, the butterfly hindwing shows expression of many genes that in *Drosophila* are repressed by *Ubx*. Thus, changes in the regulation of Ubx gene targets, through *cis*-acting elements are likely to have played a role in the evolution of the different morphologies of the dipteran and lepidopteran wing appendages.

Drosophila does not bear limbs on abdominal segments, whereas species of other hexapod orders, such as beetles, grasshoppers, and springtails have limb-bearing abdominal segments. In *Drosophila*, Distal-less (Dll), a protein required for distal limb formation, is repressed by Ubx in abdominal segments. In hexapod orders with abdominal limbs, *Dll* is expressed in trunk segments despite the presence of Ubx protein (Palopoli and Patel, 1998). This result supports the hypothesis that the interaction of *Ubx* and *Dll* evolved late in insect evolution and may have been involved in the evolution of limb specification in flies. Two recent studies also shed new light on the molecular mechanism responsible for the different Ubx functions among different species with limb bearing trunk segments. *Drosophila*, in contrast to onychophorans (Fig. 5E and F), a sister taxa of arthropods, contains an alanine-rich motif necessary for Ubx repression of *Dll* (Galant and Carroll, 2002). Another study shows that the crustacean Ubx contains both an alanine-rich motif and a second regulatory motif that modulates the function of the alanine-rich motif (Ronshaugen et al., 2002). This novel regulatory region putatively inhibits the alanine-rich motif to allow crustaceans to have limbs on trunk segments. Thus, sequential changes in Hox protein sequence that result in modulation of protein function likely played a role in evolution of limb specification in arthropods and related phyla (compare Fig. 5E and F).

Determination: blastomere potential in early mouse embryos

Determination is a classical term used to define the capacity of a cell to acquire different fates

Although the zygote has the capacity to make all the cells and tissues of a future organism (i.e., the zygote is totipotent), it is often already a highly polarised cell, in which specific

cytoplasmic domains are destined to give rise to specific tissues. For many animal species studied, the polarity of the zygote and cell-fate potential of blastomeres at the two-cell stage is already highly restricted, e.g., most insects (van Eeden and St. Johnston, 1999), nematodes (Kemphues and Strome, 1997), and amphibians (Spemann, 1938).

The mammalian embryo has always been considered unique in comparison to other vertebrate embryos, in part because the mammalian oocyte does not show an obvious polarity and in part because the individual blastomeres retain totipotency through the four-cell stage; i.e., an isolated blastomere is capable of forming a viable embryo (Tarkowski, 1959). Additionally, up to the eight-cell stage blastomeres can be removed or added without affecting viability (Gardner and Rossant, 1976) although at least a portion of both the outer and inner cells of the 16-cell mouse embryo retain totipotency (Ziomek et al., 1982) and cells of the inner cell mass remain pleuripotent even longer (Handyside, 1978; Hogan and Tilly, 1978a, 1978b; Johnson, 1979; Rossant and Lis, 1979; Spindle, 1978). In comparison, the eggs of amphibians are visible polar before fertilisation and isolated two-cell blastomeres cannot form viable embryos (Gardner and Rossant, 1976; Spemann, 1938). Despite these observations several new studies suggest that the mouse embryo is polarised at the one-cell stage. First, the plane of the first cleavage frequently occurs in the proximity of the meiotic polar body, which is tethered at the animal cap at the site of the previous meiotic division, and divides the embryo along the animal-vegetal axis (Ciemerych et al., 2000; Gardner, 1997, 2001; Piotrowska et al., 2001; Piotrowska and Zernicka-Goetz, 2001; Plusa et al., 2002). Additionally, the position of a transplanted animal cap on a developing one-cell zygote that lacks an endogenous animal cap predicts the first plane of division, but reciprocal experiments using vegetal caps do not (Plusa et al., 2002). Thus, the first cleavage plane is regulated by factors localised to the animal pole. Second, dye marked blastomeres at the two-cell stage have distinct fates. The first blastomere to divide at the two-cell stage contributes its progeny preferentially to the embryonic portion of the mouse blastocyst, whereas the tardy cell contributes its progeny preferentially to the extra-embryonic portion (Piotrowska et al., 2001). Thus, the earliest steps of embryonic development may not be so different as those of other vertebrates. Yet, despite the polarisation of the early mouse embryo, mechanisms must operate in the mammalian embryo up to the eight-cell stage that can compensate or override this built-in asymmetry to reestablish axes and cell-fate potential.

Lateral inhibition: changes in bristle patterning in Diptera

Lateral signalling is a process in which neighbouring cells inhibit each other from developing in a similar way

Sensory bristles are a basal character in dipterans (Simpson et al., 1999). Ancestrally, sensory bristle numbers are not constant and bristles are uniform in size and shape and are distributed randomly, though uniformly, over the notum. More highly evolved dipterans have more complex patterning of sensory bristles: bristles can be arranged into rows and can be classified into two phenotypic classes, microchaetes (small hair-like bristles) and macrochaetes (long stout bristles). In the most derived species, microchaetes are arranged in rows and a specific number of macrochaetes are specified in predefined locations (compare Fig. 6A and C). In *Drosophila*, microchaetes are arranged in five rows on either side of the dorsal midline and are parallel to the midline. The exact number and position of microchaetes within rows are random though the spacing between microchaetes is uniform. Additional randomly distributed, though uniformly spaced, microchaetes are present further from the midline. Macrochaetes occur in a fixed number and at characteristic sites on the notum (Fig. 6A).

Two genetic pathways have been found to be fundamental for bristle development in *Drosophila*. First, microchaetes arise from proneural domains of *achete-scute* (*ac-sc*) expression. Ac and Sc are basic helix-loop-helix transcription factors (Alonso and Cabrera, 1988; Ghysen and Dambly-Chaudiere, 1988; Gonzalez et al., 1989; Villares and Cabrera, 1987). Second, determination of the cells that will give rise to the actual bristles within the proneural cluster requires lateral signalling between the Notch transmembrane receptor and its ligand Delta (Kimble and Simpson, 1997). For microchaetes, all cells in the proneural domain initially express both Notch and Delta at comparable levels. Due to the presence of a feedback mechanism, Notch signalling results in a decrease in the amount of ligand and in *ac-sc* expression, ultimately resulting in an increase in the amount of receptor. During development, at random, a cell will express slightly more Delta ligand in comparison to Notch receptor and thus inhibit ligand expression and promote receptor expression in its neighbours. In return, due to a smaller degree of signalling from its neighbours, the same cell produces more ligand and less receptor. Thus, a series of uniformly spaced cells are chosen to become bristle cells.

Macrochaetes also rely upon *ac-sc* expression in proneural clusters and lateral inhibition by Notch. However, as the number of macrochaetes and their position on the notum is fixed, the choice of bristle precursor is biased. One way to bias bristle precursor specification is through the regulation of *ac-sc* which positively regulates the expression of *Delta* thereby resulting in bristle precursor specification (Simpson, 1997). There are four *ac-sc* genes in *Drosophila* all under complex transcriptional regulation with highly derived expression domains (Fig. 6A, diagrams) (Alonso and Cabrera, 1988; Ghysen and Dambly-Chaudiere, 1988; Gonzalez et al., 1989; Villares and Cabrera, 1987). Several genes are known that effect *ac-sc* expression; among these known genes, the GATA transcription factor *pannier* (*pnr*) and genes of the *iroquois* complex (iro-C) promote macrochaete fates, while the *extramacrochaetae* gene (*emc*) prod-

uct inhibits macrochaetes. Pnr and Araucan and Caupolican, Iro proteins, directly regulate *ac-sc* transcription (Garcia-Garcia et al., 1999; Gomez-Skarmeta et al., 1995, 1996) and are regulated in turn by *dpp* signalling (Mullor et al., 1997; Phillips et al., 1999; Sato and Saigo, 2000; Tomoyasu et al., 1998). *emc* is thought to prevent Ac and Sc binding to DNA by sequestering Ac and Sc (Cubas and Modolell, 1992; Ellis et al., 1990; Garrell and Modolell, 1990), thus preventing them from maintaining their own expression through a transcriptional feedback loop. Overlapping expression patterns of these and other genes may set up a prepattern for macrochaete position.

The evolution of the regulation of Notch lateral signalling by *ac-sc*, the subsequent control of *ac-sc* by the expression domains of selector genes such as *pnr*, the *iro-C* genes and *emc*, and the further control of these selector genes by *dpp* is likely to have resulted in the changes in bristle pattern observed in the Diptera. Recent studies on some of these genes from other dipteran species, some with more ancestral features, are providing preliminary insights into these evolutionary events (Pistillo et al., 2002; Skaer et al., 2002b; Wulbeck and Simpson, 2000, 2002). It has been hypothesized that duplication of the *ac-sc* gene complex in the dipterans was a prerequisite for the evolution of more complex expression patterns and thus bristle patterns. The *ac-sc* complex has undergone at least three duplications leading to higher dipterans and the events may correlate to increased pattern complexity (compare Fig. 6A, B, and C) (Skaer et al., 2002a). More generally, gene duplication has been proposed to be a major mechanism to allow divergence of gene function. Duplicate genes most likely start with an identical set of functions. Loss of a regulatory element in one duplicate, but not the other would lead to one gene retaining an ancestral function and the other losing it, a process called subfunctionalisation (Force et al., 1999; Hughes, 1994). Second, in a similar fashion, new elements can be acquired to permit new biological functions. Eventually each duplicate would be responsible for a disparate set of biological activities. Thus, the molecular events that result in gene duplication might be essential for providing the flexibility for the evolution of networks of interacting genes.

Gene networks: changes in wing patterning in polyphenic ant species

Genetic networks are maps that represent interactions between discrete genes and modules (cassettes of genes used to effect a common function) to execute developmental processes

The genomes of many animals and plants are able to produce different morphologies based upon environmental cues; this is known as polyphenism (Gilbert, 2002). Ants are holometabolous insects; ants, like flies, develop their wings in internal pouches, called imaginal disks, in the larvae and undergo an extensive morphogenesis where the wings invert to produce the adult appendage. Like butterflies, ants have a forewing disk and a hindwing disk. Ants exhibit wing polyphenism in the castes that make up their societies (Hölldobler and Wilson, 1990). Ant embryos can develop as a member of the worker castes, i.e., a worker or a soldier, or a member of the reproductive castes, i.e., a queen or a male, depending upon environmental cues. Worker castes generally have no or nonfunctional wings; whereas reproductive castes generally have wings. Based upon the presence of a worker caste in early ant fossils and the predominance of this caste system in extant ant species, wing polyphenism is hypothesised to have evolved only once early in ant phylogeny.

Holometabolous insects share a conserved wing patterning gene network (Carroll et al., 1994). In a recent study, Abouheif and Wray (2002) examined the expression of genes involved in patterning the wings in winged and nonwinged castes for four closely related ant species. Not surprisingly, the pattern of expression for *Ubx, extradenticle* (*exd*), *wg, scalloped* (*sd*), and *spalt* (*sal*) is conserved in winged ant castes in comparison to the model holometabolous insect *Drosophila.* Also as anticipated, the pattern of expression for individual genes involved in wing patterning is disrupted for nonwinged castes in comparison to the winged caste and *Drosophila.* Surprisingly, the expression patterns of different genes are interrupted among discrete worker castes within a species and among worker castes across ant species. Genes that are involved late in patterning are affected in some worker castes or species; i.e., in the forewing disk of *Pheidole morrisi* soldiers, *sal* expression is absent, though all the other genes examined are expressed normally. Alternatively, genes that are involved early in patterning are affected in other worker castes or species, i.e., *P. morrisi* workers exhibited no expression for any of the genes tested in the vestigial wing disks, such as *en.* In contrast, other early genes like *Ubx* and *exd* were expressed normally in the vestigial wing disks of workers from the ant species *Neoformica nitidiventris* and *Crematogaster lineolata.* This is a surprising finding because it might be anticipated that all worker castes would show common disruptions in the wing patterning gene network if wing polyphenism arose once.

The observations of Abouheif and Wray (2002) suggest two possibilities. Either polyphenism did not arise once in the ant lineage, a finding not supported by the fossil record and current phylogenies, or as the authors point out nodes of disruption in the polyphenic expression of genes involved in wing patterning are evolutionarily labile over relatively short periods of time. Rapid changes in the roles of individual genes have also been observed in other phyla, e.g., nematode gonadal and vulval morphologies (Haag and True, 2001; Rudel and Kimble, 2001, 2002). Additionally, their results suggest that phylogentic history and the disk morphology cannot accurately predict changes in the gene

network, an observation analogous to that seen in vulva formation in nematodes where distinct signalling systems and, most likely, highly modified gene networks, result in a similar vulva morphology.

Conclusions

The 10 developmental and molecular concepts used as guidelines for this review provide an excellent basis for the description and comparison of developmental processes and mechanisms. As a result, a comparison of related organisms using these concepts can be a powerful starting point to hypothesise putative evolutionary trends. However, as all of the concepts are built on developmental rather than evolutionary thinking, they must be complemented with additional evolutionary frameworks to provide insight into the evolution of development processes and to govern future experimentation in the field.

In the conclusions, we will highlight the "emerging principles" of the case studies that were discussed in the review. As a full understanding of evolutionary mechanisms requires functional genetic and genomic approaches, we will describe the need for genetic satellite systems. When complemented with accurate phylogenies, case studies in selected satellite organisms can provide molecular answers, i.e., how molecules, pathways, and mechanisms have evolved to generate the diversity of forms and structures. In this context, evolutionary frameworks will become obvious and provide insight into fascinating themes, such as parallel and convergent evolution and homoplasy, thereby presenting the future challenges of "evo-devo."

Emerging trends

Even the few comparative examples described in the context of these 10 concepts demonstrate some emerging general principles. First, fundamental autonomous cellular process, such as programmed cell death or cell migration, appear to be more complaisant for changes and cooption than complex signalling systems, e.g., monodelphic nematode gonads, cavefish eye loss, posterior nematode vulvae. Thus, cellular processes can be considered as cassettes that have been used over and over again during evolution to provide the "cellular" basis for evolutionary change. This can be compared with molecular modules, such as transcriptional regulatory modules, components of signalling cascades, or even individual domains in multidomain proteins. It will be one of the major challenges to identify if similar regulatory events are involved in the cooption of these cellular modules. Second, complex changes in gene expression patterns and signalling systems may rely heavily upon gene duplication events and changes in transcriptional activity through *cis*-regulatory elements, e.g., the activity of Hox genes in insect limb specification and insect bristle formation. However, cooption of pathways and gene duplication cannot describe the advent of all novelties. For example, a substantial number of genes within organisms with fully sequenced genomes are novel, i.e., with no substantial sequence similarity to genes from other organisms (Pires-DaSilva and Sommer, 2003). Additionally, new signalling pathways can evolve from existing pathways (Pires-DaSilva and Sommer, 2003). Although eminent as a general trend, we are far away from understanding the molecular principles of these changes. On one hand, the microevolutionary basis of such changes has to be investigated in selected examples. On the other hand, the function of fast evolving and therefore "novel" genes has to be studied (Schmid and Tautz, 1997). It is obvious that these questions are beyond the original scope of developmental biology. Therefore, a much more extensive integration of developmental and evolutionary approaches is absolutely essential.

The need for genetic satellite systems

It is obvious from all of the examples described in this review that comparisons between closely related taxa have been essential for making progress in evolutionary developmental biology. First, the homology between developmental processes among related animals makes the identification and the interpretation of differences straightforward. Second, experimental techniques and the molecular knowledge from the model system can often be used in the satellite system for detailed studies of gene function.

Although interpretable evolutionary differences can often be identified between closely related taxa, the causes behind these differences are usually not obvious and a mechanistic explanation is far from apparent. Even when a feature can theoretically be explained by a single loss-of-function mutation, i.e., a mutation in the Pax6 gene that results in no expression in the eye primordia, the "seemingly" simplest explanation is frequently not the actual explanation (Haag and True, 2001). In this example, the loss of Pax6 expression in the anterior margin of the neural plate may not reflect an actual change in the Pax6 locus, but may be due to the advent of novel signalling (Strickler et al., 2001). In other cases, morphologies appear similar with little or no difference, yet a vast amount of change is masked. The vulva in many nematodes appears morphologically similar and occurs in the same relative position along the body, but the mode of vulva specification has changed dramatically, such as differences seen between *C. elegans* and *P. pacificus* (Sommer, 2000a). Thus, even though the processes do not look perturbed, it cannot be inferred that the underlying mechanisms, the genes involved, or the functions of an individual gene have been retained. In yet another scenario, a gene might be known to have a role in a morphological change; however, we do not always know at what level the function of a gene has been altered. Changes in the function of the Hox gene Ubx have been involved in morphological

variations of limbs in insects. These changes have occurred at the level of its own expression, the targeting of downstream genes, and the modulation of its own protein function due to sequence change (Averof and Patel, 1997; Galant and Carroll, 2002; Ronshaugen et al., 2002; Weatherbee et al., 1998, 1999).

For the most part, we do not know the changes in the genomes that resulted in morphological novelties, the way these genetic changes have altered the circuitry of pathways, or how pathways are coopted to target new cassettes responsible for building new structures. To address these questions, satellite systems must be developed to allow full experimental manipulation to test gene function in vivo (Simpson, 2002). Methods for the mutagenesis of these organisms, the mapping of genetic loci, and for transformation and protein expression in these organisms must be worked out. The development of genetic and/or genomic tools has begun for several satellite systems, e.g., the nematodes *Pristionchus pacificus* (Srinivasan et al., 2001, 2002), Oscheius sp. CEW1 (Dichtel et al., 2001), the flour beetle *Tribolium castaneum* (Brown et al., 1994; Sulston and Anderson, 1996), the wasp *Nasonia vitripennis* (Pultz et al., 1999), and the fish *Astyanax mexicanus* (Borowsky and Wilkens, 2002) and *Gasterosteus aculeatus* (Peichel et al., 2001), though development of these systems are in their infancy.

Accurate phylogenies are essential for providing a meaningful context for evolution-based questions

These case studies must be framed in a phylogenetic context that is independent of developmental description. Knowledge of how individual species are related is required to know which are ancestral morphologies and which are derived morphologies, to know whether a new morphology is due to the gain or loss of a feature, and to know whether a feature has been derived once or many times. Without this phylogenetic framework, even the most basic questions cannot be framed in a meaningful way. Much progress has been made in the last decade in providing molecular phylogenies that shed new light on both, the phylogenetic relationship of major animal and plant groups, as well as on the phylogenetic relationship within individual phyla. It is mostly the latter that helps to establish the phylogenetic framework for evolutionary developmental biology. For example, the reconsideration of the relationship of the major arthropod groups with the identification of sister-group relationships of the insects and crustaceans on one hand and the chelicerates and myriapods on the other (Friedrich and Tautz, 1995; Hwang et al., 2001) was essential for interpreting developmental and morphological differences between these taxa. In a similar way, the first ever phylogeny of plant-parasitic, animal-parasitic, and free-living nematodes (Blaxter, 1998; Blaxter et al., 1998) allowed the comprehensive comparison of nematode developmental characters.

Parallel evolution, convergent evolution, and homoplasy, the challenge of the future

In an ideal experimental world for the evolutionary developmental biologist, each higher taxon contains one species, the development of which is well described at the genetic and molecular level. Several additional species exist within that taxon, which show interesting morphological and developmental differences with regard to the model organism. The phylogeny of all species is known based on morphological and molecular studies so that the direction of evolutionary change can be read by placing developmental characters onto the preexisting phylogenetic tree. Several of these species can be established as satellite systems by applying genetic and genomic tools. In such an idealistic scenario, the in-depth analysis of developmental processes among several satellite systems will not only offer answers on the evolution of developmental mechanisms, it will also provide detailed insight into general evolutionary trends and frameworks.

Although we are currently far away from such an ideal world (mostly due to technical restrictions), one recurring important theme in many of the examples described is the "convergent" evolution of a specific character in closely related but disparate phylogenetic lineages. Monodelphic gonads in nematodes, multicellularity in Volvocales, cavefish eye loss, and posterior vulvae in nematodes have all evolved several times independently within their respective clades. Our understanding of the role of parallel evolution to produce similar structures in closely related taxa and of the causes and mechanisms for the occurrence of convergent evolution is a black box; yet an understanding of how and why disparate species within groups of related organisms independently generate the same solution de novo and obtain an analogous morphology maybe the most important pursuit of all. However, the phenomena of convergence, parallelism, and homoplasy are complicated to investigate as they require detailed case studies in many closely related species and will be one of the largest challenges for the future study of the evolution of developmental mechanisms.

Even though some trends maybe found in the examples given, as evolutionary biology is a historical science that relies on the genomes in which differences are induced and the environment in which selection occurs, there are likely to be only a few emerging universal laws. Increased variation due to gene duplication and changes to regulatory and coding sequence in the genome can build up within discrete populations of a species. Many examples show that a large amount of background variation exist within populations and acts to modulate developmental processes within a species, e.g., fly haltere formation (Gibson and van Helden, 1997; Gibson et al., 1999), fly bristle specification (Takano-

Shimizu, 2000), and stickleback morphology (Ahn and Gibson, 1999; Peichel et al., 2001) to name a few. This variation may allow the creation of new gene pathways and the creation of putatively redundant or epistatic mechanisms that may be selected upon to reinforce existing networks, particularly newly created networks responsible for advantageous but unrobust developmental processes. Changes in epistatic mechanisms and compensatory changes among redundant mechanisms may result in dramatic changes to genetic networks. In our ideal world, one would want to describe in parallel all of these conditions in coexisting populations and the final evolutionary alternatives present in related "derived" species that have built redundant mechanisms and that are released from constraint. Eventually, only a complete mechanistic and molecular understanding of developmental constraints, parallel evolution, convergence, and homoplasy within discrete evolutionary model systems will bring us close to our true goal: an understanding of the causes and origins of the morphological diversity seen in present day animals, plants, and fungi.

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